

Testis abnormalities in a population of the iconic Australian species, the eastern bearded dragon *Pogona barbata*

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ABSTRACT

Testis volume is generally correlated with sperm production. Their size is, therefore, considered informative, and is widely used as a surrogate for reproductive ability in a range of species. Gonadal abnormalities that influence adult reproductive function as a result of endocrine disruption compounds (EDCs) have been reported. In response to the observation that the population of the bearded dragon *Pogona barbata* had crashed in peri-urban Western Sydney, we investigated the nature and extent of testis deformity. It was observed that individuals collected in Western Sydney since the introduction of unleaded petrol in 1985 have a high level of deformities that are consistent with disruption of the endocrine processes. While deformities, such as missing testes, extreme asymmetry between testes, and flattened testes were not associated with any single aspect of the reproductive cycle, and they were found across the range of the species, the problem was much greater in Western Sydney than elsewhere in the species' range. We suggest that the abnormalities in Western Sydney may, in part, be responsible for the crash in the local population of *P. barbata*.

Key words: endocrine disruption compounds, EDCs, testis deformity, reptile deformities, reproductive failure

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Introduction

In addition to stage of maturity or seasonal variation among adult males, a range of factors may have an influence on testis size (Awata *et al.* 2006). For example, gonadotropic hormone (GtH) is one hormone that stimulates testicular growth in squamate reptiles (Lofts 1987), and it is a seasonal regulator in other taxa, such as bears (Howell-Skalla *et al.* 2000). There may, therefore, be a range of testis sizes at any one time within a population. However, testis volume is generally correlated with sperm production (Awata *et al.* 2006; Edwards *et al.* 2006). The size of testes is therefore considered informative, and is widely used as a surrogate for reproductive ability in a range of species including birds (Rising 1996; Merilä and Sheldon 1999), reptiles (Shanbhag *et al.* 2000; Aldridge and Arackal 2005; Pizzatto 2005), and mammals (Gorman and Zucker 1995; Schoeman *et al.* 2004; Couto and Talamoni 2005).

The basic structure of the testis is regarded as being highly conserved among vertebrates (Neves *et al.* 2002) and, as a consequence, data published typically rely on the

measurement of a single testis/individual (Shea 1993; Bull *et al.* 1997; Harlow and Taylor 2000; Hernandez-Gallegos *et al.* 2002; Taylor 2004; Aldridge and Arackal 2005; Couto and Talamoni 2005; Pyter *et al.* 2005). Where directional asymmetry has been observed, there is an expectation that the condition is uniform (Rising 1996; Merilä and Sheldon 1999), but it is not always so (Graves 2004).

Environmental contaminants have been implicated in physical abnormalities of the testes in herpetofauna in the wild, for example, in the leopard frog *Rana pipiens* (Hayes *et al.* 2003), and the American alligator *Alligator mississippiensis* in the United States of America (Guillette and Iguchi 2003). In highly human-modified habits in Western Sydney, Ferraro and Burgin (1993a, b) found only a single brown-striped frog *Limnodynastes peronii* (6% of sample) with one shrunken and deformed testis, and in native habitat, a single Peron's tree frog *Litoria peronii* (0.05%) was collected with an enlarged testis. The only

other research undertaken on the herpetofauna of the area that could be considered in an assessment of reproduction was a count of the numbers of freshwater turtle hatchlings in local wetlands. While the percentage of hatchlings was extremely low compared to expectations, it was assumed that the deficit was due to nest predation (Ryan and Burgin 2007). The observation that there had been a population crash in the bearded dragon *Pogona barbata* in the same general area was also assumed to be, at least in part, due to predation (Wotherspoon 2008).

A range of environmental pollutants has been found to affect testicular form and function (Queiroz and Waissmann 2006). Although ecotoxicological studies have shown that several types of environmental chemicals are lethal to aquatic wildlife, there is growing concern that subtle, low exposure to some contaminants may cause developmental deformities (Damstra *et al.* 2002; Kloas 2002). Endocrine disrupting chemicals (EDCs) have been shown to affect the normal development of exposed individuals (Damstra *et al.* 2002) by altering the functioning of body processes (Sharpe and Skakkebaek 1993; Guillette 2000; Guillette and Gunderson 2001; Sharpe 2003), and they may also act on the reproductive endocrine system (Colborn *et al.* 1993; Crisp *et al.* 1998; Kloas 2002; Fenske *et al.* 2005). Such EDCs can enter the environment by emissions from aircraft (Merrill *et al.* 1997; Tesseraux 2004; Dietzel *et al.* 2005), motor vehicles, and volatilisation to the atmosphere (Colborn *et al.* 1993).

Baseline data for abnormalities have been gathered, together with changes in abnormality rates over time (Schoff *et al.* 2003). Abnormalities, such as retarded gonadal development (gonadal dysgenesis) and testicular oogenesis (hermaphroditism) that could ultimately influence adult reproductive function, have been found to be the result of the action of EDCs (Colborn *et al.* 1993; Hayes *et al.* 2003; Mayer *et al.* 2003), in contrast to a genetic basis for the deformity (Duijff *et al.* 2003). For example, Kassim *et al.* (1997) found that flutamide caused developmental abnormalities in rat testes, with asymmetry as one characteristic. Administration of the nonsteroidal antiandrogen flutamide to pregnant Albino Swiss (AS) laboratory rats from gestational day 10 to birth resulted in 51% non-directional asymmetry of testicular abnormality.

Fathead minnows *Pimephales promelas* exposed to EDCs in feedlot effluent were found to have reduced testis mass and loss of reproductive function (Orlando *et al.* 2004). Testis deformity in reptiles has also been related to environmental toxins of human origin (Colborn *et al.* 1993; Guillette *et al.* 1994, 1995; Semenza *et al.* 1997; Crain and Guillette 1998; Crisp *et al.* 1998; Guillette 2000). Such synthetic chemicals have been observed to cause endocrine disruption (Colborn *et al.* 1993). For example, fuels consist of a range of components (Bernabei *et al.* 2003; Dietzel *et al.* 2005), some of which are known EDCs (Reutman *et al.* 2002; Tesseraux 2004). Action on gonadal development may therefore be inhibited by manufactured compounds and/or their degradation products (de Voogt and van Hattum 2003). In their normal development through the various distinct stages of sensitivity, specifically development

of gonads and egg maturation (Colborn *et al.* 1993), EDCs can interfere with the reproduction processes of genetically viable individuals. This phenomenon is now widely acknowledged as the 'oestrogen hypothesis' (Sharpe and Skakkebaek 1993; Guillette and Gunderson 2001; Sharpe 2003), which has been proposed and refined to account for reproductive abnormalities during male development described inclusively as the testicular dysgenesis syndrome (Fisher 2004). This may be induced by increased foetal exposure to oestrogens (Sharpe 2003). In human males, shared risk factors for testicular germ cell cancer, cryptorchidism, hypospadias (Nassar *et al.* 2007), together with low sperm counts or infertility, support the suggestion that these disorders that make up testicular dysgenesis syndrome have a common origin or cause during the period of testicular or sexual differentiation (Colborn *et al.* 1993; Sharpe 2003).

Foetal exposure to EDCs has been linked to testicular cancer in men. Hardell *et al.* (2003) found that mothers with higher organochlorine (PCBs, HCB, *trans*- and *cis*-nonachlordane) levels were more likely to have given birth to sons who developed testicular cancer; and, in contrast, the sons themselves for the most part do not have elevated organochlorine levels compared with men without testicular cancer.

In areas, such as the Cumberland Plain in Western Sydney, sandwiched between the city in the East and South-east and the Blue Mountains, the plume of air pollution from the city has been identified as problematic. The area is frequently blanketed with an inversion layer rich in pollutants, including recognised EDCs (Leighton and Spark 1995). Because of its peri-urban setting, there may also be elevated levels of agricultural chemicals in the environment, and the military airport in the region may also be a source of pollution. If EDCs were in sufficient concentration in the environment, they may be contributing to the decline of one of Australia's iconic species, *Pogona barbata*. In this paper we investigate the nature and extent of testis deformity in *P. barbata* in peri-urban Western Sydney and comment on potential conservation implications. Our results are compared with *P. barbata* from elsewhere within the species range, and temporally.

Methods

Study site

Pogona barbata has a range that encompasses much of South-eastern Australia (Cogger 2000), the area of greatest human population concentration and of much of the agricultural production of the country. Species from across this range were used for comparison with individuals from peri-urban Western Sydney, on the Cumberland Plain, at the interface between the city and the Blue Mountains (a segment of the Great Dividing Range, which runs the length of the east coast of Australia). The pollution of the city of Sydney is frequently trapped over the plain due to onshore air currents moving the air inland where it gets trapped against the mountain range (EPA 2002; DEC 2004a, b).

In this area, there is relatively limited heavy industry

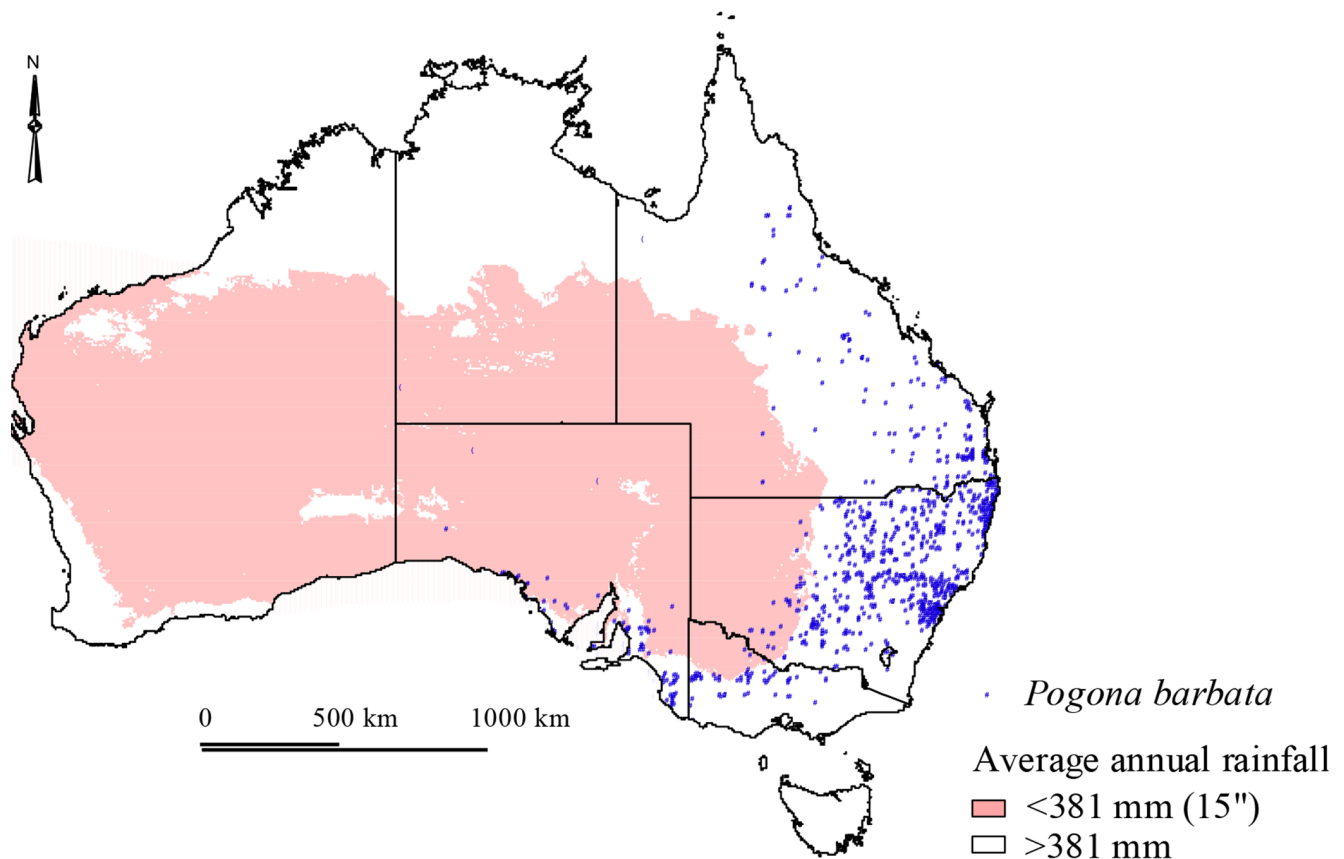


Figure 1. Distribution map for *Pogona barbata* within Australia (Source: Wotherspoon and Burgin 2011).

compared with other parts of the Sydney region. However, urbanisation is rapidly impinging on agricultural and horticultural land, and on natural bushland (DEC 2006; Beeton *et al.* 2006).

Source of animals

Animals were obtained from three sources: live collection, road kills and preserved museum specimens.

Animals sourced from road kills were collected opportunistically in Western Sydney between 1999 and 2006. These animals were maintained frozen until dissection. Live animals were also captured during this period, and ultrasound was performed on them using a high-resolution ultrasound machine (Phillips HDI 500 with a linear array 10-15 MHz transducer). Live animals were subsequently returned to the point of capture and released. These data for Western Sydney were supplemented with preserved animals from the Australian Museum, while animals sampled from across the species' range were also sourced from the Australian Museum collection.

Testis measurements and data analysis

The snout-vent length of each animal was recorded, and upon dissection or ultrasound (as appropriate) the length, width and thickness of both the left and right testes were measured to the nearest 0.1 mm with vernier callipers. No adjustment was made for shrinkage due to preservation or freezing, as it was assumed that any shrinkage would have been equivalent for both testes of the individual. Missing testes were recorded as zero values. Adjustment for testis

volume to snout-vent length was made where appropriate. Alternatively, when the comparison was between left and right testes of individual animals, or the results were expressed as a percentage of the total volume for each animal, such adjustment was not deemed necessary.

Amey (1999) observed that sexual maturity in males occurred at 133 mm snout-vent length, and dominant animals were generally greater than 200 mm snout-vent length. These groupings were accepted for this study; however, all animals with evidence of testes were sampled whether juvenile or adult.

The formula for a scalene ellipsoid was used to calculate testis volume (*cf.* Wotherspoon and Burgin 2007) for both testes for each animal. Initially an analysis of the total data set that included both testes (volume summed) for all animals was undertaken. This took the form of a regression between snout-vent length and testis volume/animal, prepared in the STATISTICA programme.

Data were also separated spatially and temporally. Since sample size was too small to allow rigorous statistical analysis by decade, data were analysed as pre- and post-1985. Although the decision was somewhat arbitrary, this specific year was ultimately chosen because it coincided with the introduction of unleaded petrol in Sydney (DEC 2006) which was heralded as being the catalyst for the reduction in air pollution in the greater metropolitan area.

The decision to divide the Western Sydney region from the rest of range was made on the basis that anecdotal evidence from members of the local herpetological society

had indicated that this population had crashed in the past 10-20 years, an observation with which the authors concurred, and was the basis of a larger study on the management of the species (Wotherspoon 2008). Analyses of sub-sets of the data were therefore undertaken to compare *P. barbata* testis condition from Western Sydney with animals from across the range, and to compare animals collected before and post-1985.

Tests for normality were determined by the Kolmogorov-Smirnov (D statistic) and Lilliefors tests (Sokal and Rohlf 1998). The Kolmogorov-Smirnov test assumes that the variable is continuous, which was confirmed. A Kruskal-Wallis test was performed to compare total testis volume for populations, temporally and spatially (Western Sydney, rest of the species range). Because the use of t-tests may be improved by assuming unequal variances (Ruxton 2006), we made that assumption and avoided a two-step test process.

A correction factor was derived to give positive numbers (where appropriate), and adjusted to a common mean of 370.9 mm³. Two outliers were removed from the data set for derivation of a line of best fit for the correction factor. Those removed were large adult males, as these may have skewed the regression line away from a simple growth relationship. Removal of those three individuals reduced the mean value by 5 mm³, but this was not a significant change. As a conservative approach, the adjustment factor used was that for the data set with outliers removed. Where considered necessary to provide a baseline of the 'normal condition', the largest testis of each animal was arbitrarily assumed to be the closest testis to that condition.

Results

The testes of 87 animals from across the range of *P. barbata* were sampled. The range in snout-vent length was 69-266 mm (mean 209 ± 31 mm), and the mean of the largest testis of each animal was 347.2 mm³ (± 255.4; range 1.6-

1165.4 mm). Some testes had greater width than length. The distribution of volume of the largest testis for each animal fitted a polynomial regression equation, and most animals were within one standard deviation of the mean. Scatter of points tended to increase with increase in the size of the animal, and was greatest among adults (Figure 2).

The differences between an individual's right and left testes were non-directionally asymmetrical, with mean -0.0636 mm (standard deviation 36.72, range -100-100 mm), and the range was not normally distributed (KS $p < 0.05$; Lilliefors $p < 0.01$) (Figure 3). The variance about the mean was not significantly different between Western Sydney and the rest of the *P. barbata* range. Individuals with the smallest size testis measurable had variation of up to 100%, and this variation occurred across the species range (Figure 4). Analysis of an individual's left and right testes as a percentage of the total volume of the testes for that individual, compared between Western Sydney and the rest of the range, pre- and post-1985, showed that there was no significant difference, spatially and temporally ($H_{3,85} = 0.749$, $p = 0.862$).

Analysis of the largest testis of an animal adjusted for snout-vent length from Western Sydney, compared spatially and temporally, showed a highly significant difference ($H_{3,87} = 10.644$, $p = 0.14$). This difference was largely due to the difference in size of the largest testis in animals from pre- and post-1985 captured in Western Sydney ($p = 0.018$, other two-way comparisons, $p = 0.106-1.00$). The mean largest testis volume of animals collected post-1985 in Western Sydney was smaller than those collected pre-1985, and smaller than animals collected from elsewhere in the species range (Figure 5). When the same analysis was undertaken on an individual's combined testis volume, the same pattern of statistical significance emerged ($H_{3,85} = 12.649$, $p = 0.006$, comparison pre- and post-1985 Western Sydney $p = 0.004$, other two-way comparisons, $p = 0.082-1.00$).

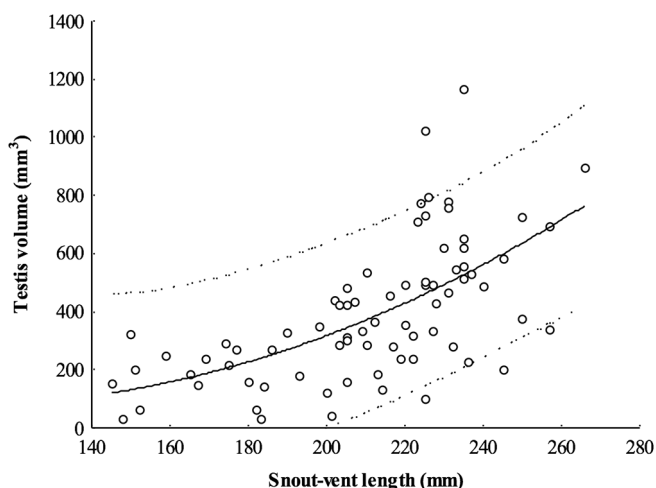


Figure 2. Regression with 95% confidence intervals for snout-vent length against calculated volume of largest testis of individual *Pogona barbata* from across their range in Southeastern Australia (polynomial regression equation = $159.2 - 3.5 \times x + 0.0216 \times x^2$).

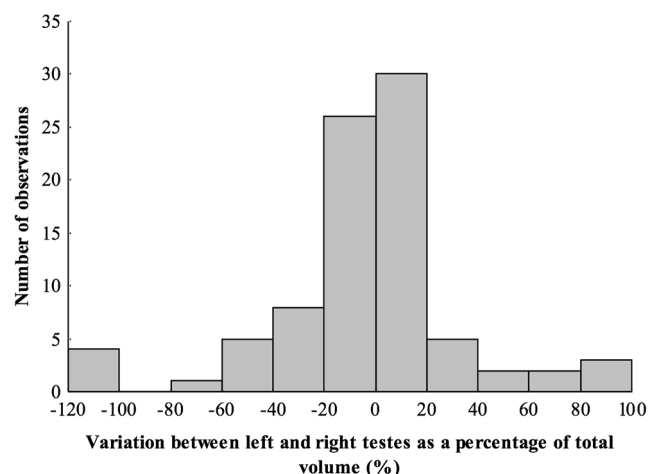


Figure 3. Difference between left and right testes of *Pogona barbata* as a percentage of total volume of the testes for each animal. Animals sampled from across the range of the species in South-eastern Australia.

Of the 14 males collected from Western Sydney after 1985, one from 1987 had 'normal' testes. The other individuals were captured between 1999 and 2006. Eleven of these showed signs of abnormality: 10 had a deviation in testis size of greater than 20%, two were missing both testes, and overall, five had at least one testis flattened, even those caught in the breeding season. This indicated an overall abnormality rate in Western Sydney over the previous decade of 76.9%.

Discussion

No previous studies have addressed reproductive fitness of *P. barbata* in the Sydney region despite anecdotal evidence that they have declined across the area (White and Burgin 2004). Explanations have generally been associated with predation, particularly by foxes which have been reported to destroy over 90% of freshwater turtle nests in the inland Murray-Darling River Basin of South-eastern Australia (Thompson 1999), and they also are nest predators in the Sydney Region (Burgin 2006). In addition to natural predators, introduced species (e.g., domestic dogs and cats) also prey on skinks (Barratt 1997; Koenig *et al.* 2002; Paltridge 2002), and presumably also small dragons. Other factors that contribute to the loss of *P. barbata* from the wild are road kills which tend to remove the largest males and, although illegal, collection of individuals for pets (Wotherspoon 2008). Although there is no previous evidence that reproductive failure may be a factor in the decline of the species, we consider that reduced fertility would be contributing to their demise.

In Western Sydney, *P. barbata* males caught post-1985 had smaller gonads (adjusted for animal size) than elsewhere in the range. This phenomenon is recent. Animals collected in Western Sydney prior to 1985 had testes that were broadly equivalent to those of individuals from elsewhere in their range. In contrast, most animals collected from Western Sydney within the

last decade had testis deformities that varied between substantial asymmetry to a total lack of functional testes. These data show a wide variation for an organ that is regarded as being highly conserved in reptiles (Greenville and Dickman 2005). However, asymmetry of $\leq 20\%$ has been observed in other species (Calhim and Birkhead 2007), and adolescent boys frequently have 15%, and occasionally more than 30%, difference in testis volume during testicular development (Thomas and Elder 2002). In a sample of 45 bird species, the average testis asymmetry was found to be 17% (Calhim and Birkhead 2007). In contrast, species such as the skink *Lerista labialis* showed no asymmetry (Greenville and Dickman 2005). Conversely, effectively half of the *P. barbata* we sampled had greater than 20% non-directional asymmetry. This indicates that substantial asymmetry was occurring across the range of the species and, on occasions, only one testis was present.

Testicular volumes may change seasonally in many species (Edwards *et al.* 2006). In birds, where testicular asymmetry occurs, it is a seasonal phenomenon that develops with the age of the animal (Graves 2004). However, the pattern of variation we encountered did not fit a seasonal pattern since males were found with asymmetrical, flattened, and/or shrunken testes throughout the year. There are no other data available that would explain that the sometimes dramatic deviation from unity was a 'normal' condition over such a large proportion of a population.

Functional fertility is directly related to testicular volume (Moller 1988, 1991; Birkhead *et al.* 1994; Sheldon 1994). Our results indicate that, under even the most conservative assessment of the reproductive fitness of the Western Sydney population of *P. barbata*, there has been a decline. While indications are that non-directional asymmetry is the normal condition, the numbers of individuals with gross deformities of the testes has increased (e.g., missing, shrunken or flattened testes), especially over

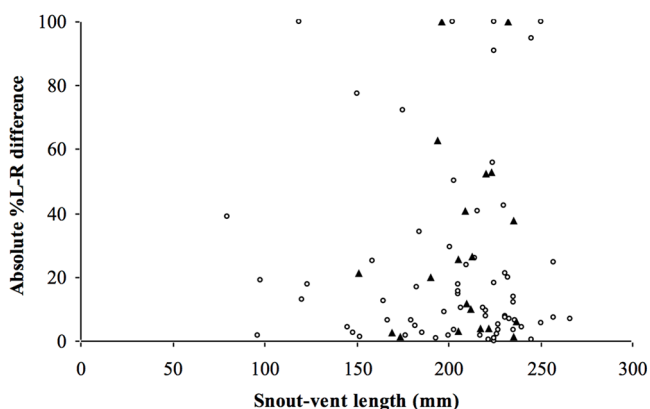


Figure 4. Differences between left and right testes of *Pogona barbata* as a percentage of total volume of testes for each animal compared to snout-vent length. Animals sampled from across the range of the species in Southeastern Australia. Triangles represent sample from Western Sydney, open circles represent individuals from the rest of the species range.

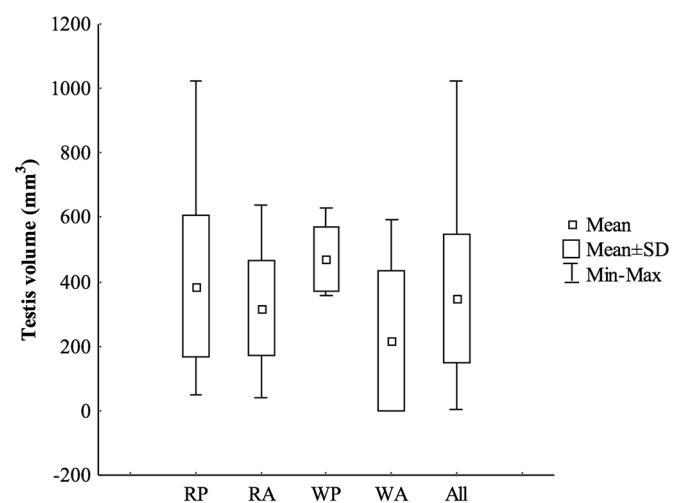


Figure 5. Box plot of variation between *Pogona barbata* collected from Western Sydney compared to the rest of the range of the species in Southeastern Australia, compared between pre- and post-1985. RP = rest of range prior to 1985, RA = rest of range from 1985, WP = Western Sydney prior to 1985, WA = Western Sydney from 1985.

the last decade, and while the level of recent deformities was greatest in Western Sydney, there was a trend that indicated that the problem was across the geographic range of *P. barbata*.

Recent surveys of natural amphibian populations have shown correlations between population decline and proximity to agricultural lands (Bishop *et al.* 1999; Relyea 2005). Water is a primary medium by which pesticides are transported from the site of application to adjacent lands (Bakouri *et al.* 2005). Spray drift and edge-of-field runoff (Schulz *et al.* 2001a, b; Dabrowski *et al.* 2005) are the major mechanisms for transport of pesticides from agricultural environments into non-target environments (Schulz 2000). This may be a mechanism that was operating in Western Sydney; however, farm chemical use is likely to be more targeted in the small-scale food producing (e.g., vegetables and fruit) properties of Western Sydney than in areas of broad-scale cropping elsewhere in the species range, that in places use extensive aerial spraying.

Air quality of Western Sydney deteriorated in the 1980s (EPA 2002; DEC 2004a, b), with an atmospheric pattern that concentrated a range of organic compounds either known as, or likely to be EDCs (Hyde *et al.* 1978; SPCC 1986; Nelson 1993; Leighton and Spark 1995). One response to this phenomenon was the introduction of unleaded petrol (DEC 2006). There is no apparent evidence from our results that the poor air quality prior to the introduction of unleaded petrol was detrimental to the reproductive health of *P. barbata*. However, some newly introduced compound/s or mix of chemicals into the environment, for example, associated with agriculture, the air pollution mix, and/or irrigation water (recycled effluent is being increasingly used in the area) has made a major change to, at least, the reproductive health of *P. barbata*, and probably at least some other animal species of the area.

A range of potential or identified EDCs (Colborn *et al.* 1993) has been recorded in measurable concentrations over Western Sydney since at least 1996 (EPA 2002; DEC 2004a, b). Dioxins, benzene, 1,3-butadiene, benzo (a) pyrene and other polycyclic aromatic hydrocarbons occur locally in elevated concentrations. Winter conditions in Western Sydney tend to reduce mixing in the atmosphere because of stronger and more frequent temperature inversions. As a result, pollutants can be trapped in a shallow layer at ground level and concentrated. This is often compounded by calm conditions, further limiting dispersal of the pollutants from over the suburbs of Western Sydney, (e.g., dioxins – Westmead, winter average, 1996-2003 480 fg/m³, maximum 1,500 fg/m³; benzene - St Marys, 1996-2003 approximately 0.3 ppbV ppb by volume; polycyclic aromatic hydrocarbons – Blacktown, winter average 1997-2001 4.29 ng/m³, maximum of 14.3 ng/m³; Benzo (a) pyrene – Blacktown, 1997-2001 winter average 0.43 ng/m³, maximum 1.92 ng/m³). The background levels, for example of dioxins, are an order of magnitude greater than found in rural Central-western New South Wales and New Zealand (EPA 2002). Despite these elevated levels of potential EDCs, it does not explain the great disparity in abnormality levels observed between Western Sydney and the rest of the species' range which includes

much of the most urbanised areas of Australia. The air pollution of the local area could also be exacerbated by air traffic associated with the local military airbase which would introduce the products of aviation fuel and de-icing residues. However, this base has been in place since 1937, and has previously had higher volumes of air traffic than currently occurs. Another source of potential EDCs (e.g. xeno-compounds) includes irrigation from recycled effluent used locally, which may include volatile EDCs. While, as Manning (2005) suggested, there may be a link between these compounds and the deformities observed, there is no obvious reason why this problem should be worst in Western Sydney or affect a terrestrial species that does not come in contact with these waters.

Some EDCs may be taken up by plants (Doucette *et al.* 2005), and legumes such as introduced clovers are high in phytoestrogens (Saloniemi *et al.* 1995). Clover constitutes a substantial component of the adult male diet, and to a lesser extent the female diet. Males, in particular, may therefore obtain EDCs in their diet. The major dietary component of *P. barbata* is invertebrates, and juveniles are fully insectivorous (Wotherspoon and Burgin, in review). This prey could bioaccumulate phytoestrogens. Under such a scenario, embryos could be exposed to maternally-derived chemical exposure during early embryonic development (Damstra *et al.* 2002; Yellayi *et al.* 2002; Selvaraj *et al.* 2004); however, it is unlikely to be the primary cause of the problem. This is because exotic legumes and clovers have been widely used in agriculture throughout Australia, and have become naturalised as weeds over the full range of *P. barbata*, and there was no indication that only animals from Western Sydney consumed a diet high in these exotic species.

Conclusions

There has been limited reporting of the effects of EDCs on gonadal development and subsequent fecundity; however, difference in testis size is established early in development (Kloas 2002). Since the pattern found in *P. barbata* did not appear to change with age, it appears that the trigger is operating in the embryonic stage, as previously observed in humans (Hardell *et al.* 2003), amphibians (Kloas 2002), and some other vertebrate classes (Edwards *et al.* 2006).

Previous researchers (Colborn *et al.* 1993; Sharpe 2003; Edwards *et al.* 2006) have confirmed that testicular abnormalities have a common origin or cause in the period of testicular or sexual differentiation. Sharpe (2003) suggested that potent oestrogens are powerful anti-androgens and inhibitors of normal reproductive tract development in males. As this process occurs during embryonic development (Edwards *et al.* 2006), for *P. barbata* it may occur within the egg while exposed to the soil environment. The EDCs may also be included during the vitellogenesis process, from EDCs accumulated in maternal fat deposits, a result of bioaccumulation from the maternal diet. The effect will not be realised until the male mates and suffers low fertility.

In Western Sydney, *P. barbata* had higher levels of structural testis abnormalities than Kassim *et al.* (1997) could induce in the laboratory in albino Swiss rats in a study of EDC

effects. Although we could not identify a single obvious factor that was responsible for the abnormalities, as a hypothesis we suggest that some form/s of EDC/s is/are responsible. One scenario that could result in levels being elevated above background levels in *P. barbata* is that the vegetation that males consume is predominantly introduced clovers (Wotherspoon and Burgin, in review), and their prey may also play a role in the bioaccumulation of the unknown compound/s. During winter, adults retreat to the canopy of trees and, at this time of year, there is frequently an inversion layer that hangs low over the woodlands, at times for substantial periods. Thus, while metabolising stored fat during winter, individuals are also potentially subjected to elevated levels of EDCs in the surrounding air. After winter they return to the woodland floor to mate with depleted fat stores and (presumably) metabolites of any toxins circulating in their blood. Females, also with diminished fat reserves, would suffer the same fate. Thus, at the time of reproductive activity and embryonic development, these reptiles are most

vulnerable to the detrimental impact of local pollutants.

Vertebrates (terrestrial and aquatic) possess similar endocrine systems and can act, to some extent, as surrogates for each other in testing or monitoring programmes (Colborn *et al.* 1993; Shi 1999; Miyamoto and Burger 2003; Tata 2003). Due to the similarities in physiological function between reptiles and humans, the abnormalities in *P. barbata* may have implications for humans (Ballatori and Villalobos 2002). However, unlike mammals, Australian agamids may have either temperature-dependent or genotypic sex, and multiple transitions have apparently occurred between these sex-determining mechanisms (Azaz *et al.*, 2009). The ability to switch in this manner demonstrates that, unlike mammals (and many other animals), sex determination in this group is somewhat labile (Azaz *et al.*, 2009). For this reason *P. barbata* may be much more vulnerable to the impact of EDCs than humans. Our findings may, therefore, be an early warning of a potentially wider spread problem.

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References

- Aldridge, R.D. and Arackal, A.A. 2005. Reproductive biology and stress of captivity in male brown treesnakes (*Boiga irregularis*) on Guam. *Australian Journal of Zoology* 53: 249-256. <http://dx.doi.org/10.1071/ZO05010>
- Ame, A.P. 1999. Reproductive Biology of the Bearded Dragon, *Pogona barbata* (Cuvier, 1829). Ph.D Thesis, University of Queensland, Brisbane.
- Awata, S., Heg, D., Munehara, H. and Kohda, M. 2006. Testis size depends on social status and the presence of male helpers in the cooperatively breeding cichlid *Julidochromis ornatus*. *Behavioural Ecology* 17: 372-379. <http://dx.doi.org/10.1093/beheco/arj043>
- Bakouri, H.E., Palacios-Santander, J.M., Cubillana-Aguilera, L., Ouassini, A., Naranjo-Rodriguez, I. and Hidalgo-Hidalgo de Cisneros, J.L. 2005. Electrochemical analysis of endosulfan using a C18- modified carbon-paste electrode. *Chemosphere* 10: 565-571.
- Ballatori, N. and Villalobos, A. R. 2002. Defining the molecular and cellular basis of toxicity using comparative models. *Toxicology and Applied Pharmacology* 183: 207-220. <http://dx.doi.org/10.1006/taap.2002.9488>
- Barratt, D.G. 1997. Predation by house cats, *Felis catus* (L.), in Canberra, Australia. I. Prey composition and preference. *Wildlife Research* 24: 263-277. <http://dx.doi.org/10.1071/WR96020>
- Beeton, R.J.S., Buckley, K.I., Jones, G.J., Morgan, D., Reichelt, R.E. and Trewin, D. 2006. Australia State of the Environment 2006, Independent Report to the Australian Government Minister for the Environment and Heritage, Department of the Environment and Heritage, Canberra.
- Bernabei, M., Reda, R., Galiero, R. and Bocchinfuso, G. 2003. Determination of total and polycyclic aromatic hydrocarbons in aviation jet fuel. *Journal of Chromatography, A* 985: 197-203. [http://dx.doi.org/10.1016/S0021-9673\(02\)01826-5](http://dx.doi.org/10.1016/S0021-9673(02)01826-5)
- Birkhead, T.R., Veiga, J.T. and Moller, A.P. 1994. Male sperm reserves and copulation behaviour in the house sparrow. *Proceedings of Royal Society London B* 256: 247-251. <http://dx.doi.org/10.1098/rspb.1994.0077>
- Bishop, C. A., Mahony, N. A., Struger, J., Ng, P. and Pettit, K. E. 1999. Anuran development, density, and diversity in relation to agricultural activity in the Holland River watershed, Ontario, Canada (1990-1992). *Environmental Monitoring and Assessment* 57: 21-43. <http://dx.doi.org/10.1023/A:1005988611661>
- Bull, K.H., Mason, R.T. and Whittier, J. 1997. Seasonal testicular development and sperm storage in tropical and subtropical populations of the brown tree snake (*Boiga irregularis*). *Australian Journal of Zoology* 45: 479-488. <http://dx.doi.org/10.1071/ZO97027>
- Burgin, S. 2006. Confirmation of an established population of exotic turtles in urban Sydney. *Australian Zoologist* 33: 379-384. <http://dx.doi.org/10.7882/AZ.2006.011>
- Calhim, S. and Birkhead, T.R. 2007. Testes size in birds: quality versus quantity--assumptions, errors, and estimates. *Behavioural Ecology* 18: 271-275. <http://dx.doi.org/10.1093/beheco/arl076>
- Cogger, H.G. 2000. Reptiles and Amphibians of Australia. Sixth Edition. Reed New Holland [Australia] Pty Ltd, Sydney.
- Colborn, T., vom Saal, F.S. and Soto, A.M. 1993. Developmental effects of endocrine-disrupting chemicals in wildlife and humans. *Environmental Health Perspectives* 102: 378-384. <http://dx.doi.org/10.1289/ehp.93101378>
- Couto, D. and Talamoni, S.A. 2005. Reproductive condition of *Akodon montensis* (Thomas) and *Bolomys lasiurus* (Lund) (Rodentia, Muridae) based on histological and histometric

- analyses of testes and external characteristics of gonads. *Acta Zoologica* 86: 111-118. <http://dx.doi.org/10.1111/j.1463-6395.2005.00191.x>
- Crain, D.A. and Guillette, L.J.Jr. 1998. Reptiles as models of contaminant-induced endocrine disruption. *Animal Reproduction Science* 53: 77-86. [http://dx.doi.org/10.1016/S0378-4320\(98\)00128-6](http://dx.doi.org/10.1016/S0378-4320(98)00128-6)
- Crisp, T.M., Clegg, E.D., Cooper, R.L., Morrow, M.S., Rodier, D.J., Schaeffer, J.E., Touart, L.W., Zeeman, M.G. and Patel, Y.M. 1998. Environmental disruption: an effects assessment and analysis. *Environmental Health Perspectives* 106 (Supplement 1): 11-56. <http://dx.doi.org/10.1289/ehp.98106s111>
- Dabrowski, J.M., Bollen, A. and Schulz, R. 2005. Combined effects of discharge, turbidity, and pesticides on mayfly behavior: Experimental evaluation of spray-drift and runoff scenarios. *Environmental Toxicology and Chemistry* 24: 1395-1402. <http://dx.doi.org/10.1897/04-222R.1>
- Damstra, T., Barlow, S., Bergman, A., Kavlock, R. and Van Der Kraak, G. 2002. Global Assessment of the State-of-the-Science of endocrine disruptors. International Programme on Chemical Safety. WHO/PCS/EDC/02.2, World Health Organization, Geneva.
- DEC 2004a. Ambient Air Quality Research Project (1996-2001): Internal Working Paper No. 2, Ambient Concentrations of Toxic Organic Compounds in NSW. Department of Environment and Conservation, Sydney. <http://www.environment.nsw.gov.au/resources/air/organics04120.pdf>
- DEC 2004b. Ambient Air Quality Research Project (1996-2001): Internal Working Paper No. 3, Ambient Concentrations of Polycyclic Aromatic Hydrocarbon Species in NSW. Department of Environment and Conservation, Sydney. <http://www.environment.nsw.gov.au/resources/air/pah.pdf>
- DEC 2006. State of the environment report. Department of Environment and Conservation, Sydney. https://www.google.com/search?q=state+of+the+environment+report+2006&hl=en&spell=1&oq=State+of+the+environment+report+2006&gs_l=heirloom-serp.1.0.0i22i30l8.2351.17801.0.20531.67.18.0.0.0.5.260.2380.0j3j8.11.0.ekp%2Ckpn%3D1000%2Ckpn%3D100...0...1.34.heirloom-serp..62.5.1130.Xe6o6esb-g8
- de Voogt, P. and van Hattum, B. 2003. Critical factors in exposure modelling of endocrine active substances. *Pure and Applied Chemistry* 75: 1933-1948. <http://dx.doi.org/10.1351/pac200375111933>
- Dietzel, K.D., Campbell, J.L., Bartlett, M.G., Witten, M.L. and Fisher, J.W. 2005. Validation of a gas chromatography/mass spectrometry method for the quantification of aerosolized Jet Propellant 8. *Journal of Chromatography A* 1093: 11-20. <http://dx.doi.org/10.1016/j.chroma.2005.07.049>
- Doucette, W.J., Wheeler, B.R., Chard, J.K. and Bugbee, B. 2005. Uptake of nonylphenol and nonylphenol ethoxylates by crested wheatgrass. *Environmental Toxicology and Chemistry* 24: 2965-2973. <http://dx.doi.org/10.1897/05-171R.1>
- Duijf, P.H.G., van Bokhoven, H. and Brunner, H.G. 2003. Pathogenesis of split-hand/split-foot malformation. *Human Molecular Genetics* 12: R51-R60. <http://dx.doi.org/10.1093/hmg/ddg090>
- Edwards, T.M., Moore, B.C. and Guillette, L.J.Jr. 2006. Reproductive dysgenesis in wildlife: A comparative view. *International Journal of Andrology* 29: 109-121. <http://dx.doi.org/10.1111/j.1365-2605.2005.00631.x>
- EPA 2002. Ambient Air Quality Research Project (1996-2001): Dioxins, Organics, Polycyclic Aromatic Hydrocarbons and Heavy Metals. Environment Protection Authority, Sydney.
- Ezaz, T., Quinn, A.E., Sarre, S.D., O'Meally, D., Georges, A. and Marshall Graves, J.A. 2009. Molecular marker suggests rapid changes of sex-determining mechanisms in Australian dragon lizards. *Chromosome Research* 17: 91-98. <http://dx.doi.org/10.1007/s10577-008-9019-5>
- Fenske, M., Maack, G., Schafers, C., and Segner, H. 2005. An environmentally relevant concentration of estrogen induces arrest of male gonad development in zebrafish, *Danio rerio*. *Environmental Toxicology and Chemistry* 24: 1088-1098. <http://dx.doi.org/10.1897/04-096R1.1>
- Ferraro, T.J., and Burgin, S. 1993a. Amphibian decline: A case study in western Sydney. Pp. 197-204 in *Herpetology in Australia: A Diverse Discipline* edited by Lunney, D. and Ayers, D. Royal Zoological Society of New South Wales, Mosman.
- Ferraro, T.J., and Burgin, S. 1993b. Review of environmental factors influencing the decline of Australian frogs. Pp. 205-218 in *Herpetology in Australia: A Diverse Discipline* edited by Lunney, D. and Ayers, A. Royal Zoological Society of New South Wales, Mosman.
- Fisher, J.S. 2004. Environmental anti-androgens and male reproductive health: focus on phthalates and testicular dysgenesis syndrome. *Reproduction* 127: 305-315. <http://dx.doi.org/10.1530/rep.1.00025>
- Gorman, M.R. and Zucker, I. 1995. Seasonal adaptations of Siberian hamsters. II. Pattern of change in day length controls annual testicular and body weight rhythms. *Biology of Reproduction* 53: 116-125. <http://dx.doi.org/10.1095/biolreprod53.1.116>
- Graves, G.R. 2004. Testicular volume and asymmetry are age-dependent in black-throated blue warblers (*Dendroica caerulescens*). *The Auk* 121: 473-485. <http://dx.doi.org/10.2307/4090411>
- Greenville, A.C. and Dickman, C.R. 2005. The ecology of *Lerista labialis* (Scincidae) in the Simpson Desert: Reproduction and diet. *Journal of Arid Environment* 60: 611-625. <http://dx.doi.org/10.1016/j.jaridenv.2004.07.010>
- Guillette, L.J.Jr. 2000. Contaminant-induced endocrine disruption in wildlife. *Growth Hormone and IGF Research B* 10: 45-50. [http://dx.doi.org/10.1016/S1096-6374\(00\)80009-X](http://dx.doi.org/10.1016/S1096-6374(00)80009-X)
- Guillette, L.J.Jr., Gross, T.S., Gross, D., Rooney, A.A. and Percival, H.F. 1995. Gonadal steroidogenesis in vitro from juveniles obtained from contaminated and control lakes. *Environmental Health Perspectives* 103: 31-36. <http://dx.doi.org/10.2307/3432409>
- Guillette, L.J.Jr., Gross, T.S., Masson, G.R., Matter, J.M., Percival, H.F. and Woodward, A.R. 1994. Developmental abnormalities of the gonad and abnormal sex hormone concentrations in juvenile alligators from contaminated and control lakes in Florida. *Environmental Health Perspectives* 102: 680-688. <http://dx.doi.org/10.1289/ehp.94102680>
- Guillette, L.J.Jr. and Gunderson, M.P. 2001. Alterations in development of reproductive and endocrine systems of wildlife populations exposed to endocrine-disrupting contaminants. *Reproduction* 122: 857-864. <http://dx.doi.org/10.1530/rep.0.1220857>
- Guillette, L.J.Jr. and Iguchi, T. 2003. Contaminant-induced endocrine and reproductive alterations in reptiles. *Pure and Applied Chemistry* 75: 2275-2286.
- Hardell, L., van Bavel, B., Lindström, G., Carlberg, M., Dreifaldt, A.C., Wijkström, H., Starkhammar, H., Eriksson, M., Hallquist, A. and Kolmert, T. 2003. Increased concentrations of polychlorinated biphenyls, hexachlorobenzene,

- and chlordanes in mothers of men with testicular cancer. *Environmental Health Perspectives* 111: 930-934. <http://dx.doi.org/10.1289/ehp.5816>
- Harlow, P.S. and Taylor, J.E. 2000. Reproductive ecology of the Jacky Dragon (*Amphibolurus muricatus*): an agamid lizard with temperature-dependent sex determination. *Austral Ecology* 25: 640-652. <http://dx.doi.org/10.1111/j.1442-9993.2000.tb00070.x>
- Hayes, T., Haston, K., Tsui, M., Hoang, A., Haeffele, C. and Vonk, A. 2003. Atrazine-induced hermaphroditism at 0.1 ppb in American leopard frogs (*Rana pipiens*): Laboratory and field evidence. *Environmental Health Perspectives* 111: 568-575. <http://dx.doi.org/10.1289/ehp.5932>
- Hernandez-Gallegos, O., Mendez-do la Cruz, F.R., Villagran-Santa Cruz, M. and Andrews, R.M. 2002. Continuous spermatogenesis in the lizard *Sceloporus bicanthalis* (Sauria: Phrynosomatidae) from high elevation habitat of Central Mexico. *Herpetologica* 58: 415-421. [http://dx.doi.org/10.1655/0018-0831\(2002\)058%5B0415:CSITLS%5D2.0.CO;2](http://dx.doi.org/10.1655/0018-0831(2002)058%5B0415:CSITLS%5D2.0.CO;2)
- Howell-Skalla, L. A., Bunick, D., Nelson, R. A., and Bahr, J.M. 2000. Testicular recrudescence in the male black bear (*Ursus americanus*): Changes in testicular luteinizing hormone-, follicle-stimulating hormone, and prolactin-receptor ribonucleic acid abundance and dependency on prolactin. *Biology of Reproduction* 63: 440-447. <http://dx.doi.org/10.1095/biolreprod63.2.440>
- Hyde, R., Hawke, G.S. and Heggie, A.C. 1978. The transport and recirculation of photochemical smog across the Sydney basin. Pp. 119-134 in *Clean Air Society of Australia and New Zealand. Proceedings of International Clean Air Conference, Brisbane 15-19 May 1978*. Ann Arbor Science Publishers, Ann Arbor.
- Kassim, N.M., McDonald, S.W., Reid, O., Bennett, N.K., Gilmore, D.P. and Payne, A.P. 1997. The effects of pre- and postnatal exposure to the nonsteroidal antiandrogen flutamide on testis descent and morphology in the Albino Swiss rat. *Journal of Anatomy* 190: 577-588. <http://dx.doi.org/10.1046/j.1469-7580.1997.19040577.x>
- Kloas, W. 2002. Amphibians as a model for the study of endocrine disruptors. *International Review of Cytology* 216: 1-57.
- Koenig, J., Shine, R. and Shea, G. 2002. The dangers of life in the city: Patterns of activity, injury and mortality in suburban lizards (*Tiliqua scincoides*). *Journal of Herpetology* 36: 62-68. <http://dx.doi.org/10.2307/1565803>
- Leighton, R. and Spark, E. 1995. An Investigation into the Synoptic Patterns Associated with Air Pollution in Sydney. Environment Protection Authority, Sydney.
- Lofts, B. 1987. Testicular function. Pp. 385-409 in *Hormones and Reproduction in Fishes, Amphibians and Reptiles* edited by Norris, D.O. and Jones, R.E. Plenum Press, New York.
- Manning, T. 2005. Endocrine-disrupting chemicals: A review of the state of the science. *Australia Journal of Ecotoxicology* 11: 1-52.
- Mayer, L.P., Dyer, C.A. and Propper, C.R. 2003. Exposure to 4-tert-octylphenol accelerates sexual differentiation and disrupts expression of steroidogenic factor 1 in developing bullfrogs. *Environmental Health Perspectives* 111: 557-561. <http://dx.doi.org/10.1289/ehp.5304>
- Merilä, J.A. and Sheldon, B.C. 1999. Testis size variation in the greenfinch *Carduelis chloris*: Relevance for some recent models of sexual selection. *Behavioural Ecology and Sociobiology* 45: 115-123. <http://dx.doi.org/10.1007/s002650050545>
- Merrill, E.A., Sterner, T.R. and Larcom, B.J. 1997. Endocrine Disruptors: An Evaluation of Solvents Deicers and Jet Fuels. Government Reports Announcements and Index, No. 17. U.S. Department of Commerce, National Technical Information Service, Springfield.
- Miyamoto, J. and Burger, J. 2003. Implications of endocrine active substances for humans and wildlife. *Pure and Applied Chemistry* 75: 1617-2615.
- Moller, A.P. 1988. Testes size, ejaculate quality, and sperm competition in birds. *Biological Journal of the Linnean Society* 33: 273-283.
- Moller, A.P. 1991. Sperm competition, sperm depletion, parental care, and relative testis size in birds. *American Naturalist* 137: 882-906. <http://dx.doi.org/10.1111/j.1095-8312.1988.tb00812.x>
- Nassar, N., Bower, C. and Barker, A. 2007. Increasing prevalence of hypospadias in Western Australia, 1980-2000. *Archives of Disease in Childhood* 92: 580-584. <http://dx.doi.org/10.1136/adc.2006.112862>
- Nelson, P.F. 1993. Measurements of Toxic Compounds in Sydney's Atmosphere'. CSIRO Publishing, Sydney.
- Neves, E.S., Chiarini-Garcia, H. and Franca, L.R. 2002. Comparative testis morphometry and seminiferous epithelium cycle length in donkeys and mules. *Biology of Reproduction* 67: 247-255. <http://dx.doi.org/10.1095/biolreprod67.1.247>
- Orlando, E.F., Kolok, A.S., Binzick, G.A., Gates, J.L., Horton, M.K., Lambright, C.S., Gray, L.E., Soto, A.M. and Guillelte, L.J.Jr. 2004. Endocrine-disrupting effects of cattle feedlot effluent on an aquatic sentinel species, the fathead minnow. *Environmental Health Perspectives* 112: 353-358. <http://dx.doi.org/10.1289/ehp.6591>
- Paltridge, R. 2002. The diets of cats, foxes and dingoes in relation to prey availability in the Tanami Desert, Northern Territory. *Wildlife Research* 29: 389-403.
- Pizzatto, L. 2005. Reproductive biology of the glass snake *Ophiodon fragilis* (Squamata : Anguillidae) in South-east Brazil. *Journal of Herpetology* 15: 9-13.
- Pyter, L.M., Hotchkiss, A.K. and Nelson, R.J. 2005. Photoperiod-induced differential expression of angiogenesis genes in testes of adult *Peromyscus leucopus*. *Reproduction* 129: 201-209. <http://dx.doi.org/10.1530/rep.1.00448>
- Queiroz, E.K. and Waissmann, W. 2006. Occupational exposure and effects on the male reproductive system. *Cadernos de Saúde Pública* 22: 485-493. <http://dx.doi.org/10.1590/S0102-311X2006000300003>
- Relyea, R.A. 2005. The lethal impacts of roundup and predatory stress on six species of North American tadpoles. *Archives Environmental Contamination and Toxicology* 48: 351-357. <http://dx.doi.org/10.1007/s00244-004-0086-0>
- Reutman, S.R., LeMasters, G.K., Knecht, E.A., Shukla, R., Lockey, J.E., Burroughs, G.E. and Kesner, J.S. 2002. Evidence of reproductive endocrine effects in women with occupational fuel and solvent exposures. *Environmental Health Perspectives* 110: 805-811. <http://dx.doi.org/10.1289/ehp.02110805>
- Rising, J.D. 1996. Relationship between testis size and mating systems in American sparrows (Emberizinae). *The Auk* 113: 224-228. <http://dx.doi.org/10.2307/4088950>
- Ruxton, G.D. 2006. The unequal variance t-test is an underused alternative to Student's t-test and the Mann-Whitney U test. *Behavioural Ecology* 17: 688-690. <http://dx.doi.org/10.1093/beheco/ark016>
- Ryan, M. and Burgin, S. 2007. Status report on *Trachemys scripta elegans*: Pet terrapin or Australia's pest turtles? Pp. 1-7 in *Pest or Guest: The Zoology of Overabundance* edited by Lunney,

- D., Hutchings, P., Eby, P. and Burgin, S. Royal Zoological Society of New South Wales, Mosman.
- Saloniemi, H., Wahala, K., Nykanen-Kurki, P. and Kallela, K., Saastamoinen, I. 1995. Phytoestrogen content and estrogenic effect of legume fodder. *Proceedings of the Society for Experimental Biology and Medicine* 208: 13-17. <http://dx.doi.org/10.3181/00379727-208-43825>
- Schoeman, S., Bennett, N. C., van der Merwe, M. and Schoeman, A.S. 2004. Aseasonal reproduction in the Hottentot golden mole, *Amblysomus hottentotus* (Afrosoricida: Chrysochloridae) from KwaZulu-Natal, South Africa. *African Zoology* 39: 41-46.
- Schoff, P.K., Johnson, C.M., Schotthoefer, A.M., Murphy, J.E., Lieske, C., Cole, R.A., Johnson, L.B. and Beasley, V.R. 2003. Prevalence of skeletal and eye malformations in frogs from north-central United States: Estimations based on collections from randomly selected sites. *Journal of Wildlife Diseases* 39: 510-521. <http://dx.doi.org/10.7589/0090-3558-39.3.510>
- Schulz, R. 2000. Rainfall induced sediment and pesticide input from orchards into the Lourens river, Western Cape, South Africa: Importance of a single event. *Water Research* 35: 1869-1876. [http://dx.doi.org/10.1016/S0043-1354\(00\)00458-9](http://dx.doi.org/10.1016/S0043-1354(00)00458-9)
- Schulz, R., Peall, S.K.C., Dabrowski, J.M. and Reinecke, A.J. 2001a. Current-use insecticides, phosphates and suspended solids in the Lourens River, Western Cape, during the first rainfall event of the wet season. *Water SA* 27: 65-70.
- Schulz, R., Pell, S.K.C., Dabrowski, J.M. and Reinecke, A.J. 2001b. Spray deposition of two insecticides into surface waters in a South African orchard area. *Journal of Environmental Quality* 30: 814-822. <http://dx.doi.org/10.2134/jeq2001.303814x>
- Selvaraj, V., Zakroczymski, M.A., Naaz, A., Mukai, M., Ju, Y.H., Doerge, D.R., Katzenellenbogen, J.A., Helferich, W.G. and Cooke, P.S. 2004. Estrogenicity of the isoflavone metabolite equol on reproductive and non-reproductive organs in mice. *Biology of Reproduction* 71: 966-972. <http://dx.doi.org/10.1095/biolreprod.104.029512>
- Semenza, J.C., Tolbert, P.E., Rubin, C.H., Guillette, L.J.Jr. and Jackson, R J. 1997. Reproductive toxins and alligator abnormalities at Lake Apopka, Florida. *Environmental Health Perspectives* 105: 1030-1032. <http://dx.doi.org/10.1289/ehp.971051030>
- Shanbhag, B.A., Radder, R.S. and Saidapur, S.K. 2000. GnRH but not warm temperature induces recrudescence of quiescent testes in the tropical lizard *Calotes versicolor* (Daud.) during postbreeding phase. *General and Comparative Endocrinology* 119: 232-238.
- Sharpe, R.M. 2003. The 'oestrogen hypothesis' - where do we stand now? *International Journal of Andrology* 26: 2-15. <http://dx.doi.org/10.1046/j.1365-2605.2003.00367.x>
- Sharpe, R.M. and Skakkebaek, N.E. 1993. Are oestrogens involved in falling sperm counts and disorders of the male reproductive tract? *Lancet* 341: 1392-1395. [http://dx.doi.org/10.1016/0140-6736\(93\)90953-E](http://dx.doi.org/10.1016/0140-6736(93)90953-E)
- Shea, G.M. 1993. The male reproductive cycle of the eastern blue-tongued lizard *Tiliqua scincoides scincoides* (Squamata: Scincidae) Pp. 397-403 in *Herpetology in Australia: A Diverse Discipline* edited by Lunney, D. and Ayers, D. Royal Zoological Society of New South Wales, Mosman.
- Sheldon, B.C. 1994. Male phenotype, fertility, and the pursuit of extra-pair copulations in female birds. *Proceedings of the Royal Society of London*, B. 257: 24-30.
- Shi, Y.-B. 1999. *Amphibian Metamorphosis: From Morphology to Molecular Biology*. John Wiley, New York.
- Sokal, R.R. and Rohlf, F.J. 1998. *Biometry: The Principles and Practice of Statistics in Biological Research*. W.H. Freeman and Company, New York.
- SPCC 1986. *Report on Polycyclic Aromatic Hydrocarbons in Sydney, Port Kembla and Newcastle Air*. State Pollution Control Commission, Sydney.
- Tata, T.R. 2003. Hormonal signalling during amphibian metamorphosis. *Proceedings of the Indian National Academy of Science B*. 69: 773-790.
- Taylor, J.E. 2004. Reproduction in sympatric lizards: comparison of two species of *Ctenotus* (Scincidae) in South-eastern Australia. *Australian Journal of Zoology* 52: 649-666.
- Tesseraux, I. 2004. Risk factors of jet fuel combustion products. *Toxicology Letters* 149: 295-300. <http://dx.doi.org/10.1016/j.toxlet.2003.12.040>
- Thomas, J.C. and Elder, J.S. 2002. Testicular growth arrest and adolescent varicocele: does varicocele size make a difference? *Journal of Urology* 168: 1689-1691. <http://dx.doi.org/10.1097/00005392-200210020-00007>
- Thompson, M.B. 1999. Populations of the Murray River tortoise, *Emydura* (Chelodina): the effect of egg predation by the red fox, *Vulpes vulpes*. *Australian Wildlife Research* 10: 363-371. <http://dx.doi.org/10.1071/WR9830363>
- White, A.W. and Burgin, S. 2004. Current status and future prospects of reptiles and frogs in Sydney's urban-impacted bushland reserves Pp. 109-123 in *Urban Wildlife: More than Meets the Eye* edited by Lunney, D. and Burgin, S. Royal Zoological Society of New South Wales, Mosman.
- Wotherspoon, A.D. 2008. *Ecology and Management of Eastern Bearded Dragon Pogona barbata*. Ph.D Thesis, University of Western Sydney, Penrith.
- Wotherspoon, D. and Burgin, S. 2007. Lizard testis volume measurements: are they always underpinned by the correct assumptions? *European Journal of Anatomy* 11: 163-167.
- Wotherspoon, D. and Burgin, S. 2011. Allometric variation among juvenile, adult male and female eastern bearded dragons *Pogona barbata* (Cuvier, 1829), with comments on the behavioural implications. *Zoology* 114: 23-28. <http://dx.doi.org/10.1016/j.zool.2010.09.003>
- Wotherspoon, D. and Burgin, S. in review. Ontogenetic dietary shift in *Pogona barbata*, the Australian eastern bearded dragon. *Animal Biology*.
- Yellayi, S., Naaz, A., Szewczykowski, M.A., Sato, T., Woods, J.A., Chang, J., Segre, M., Allred, C.D., Helferich, W.G. and Cooke, P.S. 2002. The phytoestrogen genistein induces thymic and immune changes: A human health concern? *PNAS* 99: 7616-7621. <http://dx.doi.org/10.1073/pnas.102650199>

APPENDIX I



Twenty-five years ago it was a common sight to see *Pogona barbata* on fence posts in peri-urban Sydney. Now it is an uncommon occurrence. Photographer John Spradbrow.



'All talk and no action'? The reproductive capability of many male Eastern Bearded Dragon *Pogona barbata* in Western Sydney is now compromised due to testis abnormalities. Photographer Geoff Higgins.



Pogona barbata retreat to the upper branches of woodland trees to sit out the winter. Temperature inversions over substantial time periods result in these perching sites being subjected to elevated levels of air pollution contained within the fog. Photographer Danny Wotherspoon.

APPENDIX I



Gravid female *Pogona barbata* digging a nest to lay her eggs. Although the process of endocrine disruption occurs throughout development of the egg, much of it occurs during the development of the embryo in the nest. Photographer unknown.



Successful hatching of *Pogona barbata* but has this hatchling's reproductive capacity already been compromised? Photographer Danny Wotherspoon.



Bill Wotherspoon introducing grandson Bradley to *Pogona barbata* at Caniaba, near Lismore (New South Wales). This iconic species was once widespread across Eastern Australia but is suffering decline, most dramatically in Western Sydney. Photographer Betty Wotherspoon.